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Mitochondrial complementation: a possible neglected factor behind early eukaryotic sex

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Code availability

The full R code running the model and producing the figures is available freely at: https://github.com/Redsiana/Escape-from-mitochondria

Authors' contributions

A.T. designed the project and implemented the model, A.T., J.R.C. and H.K. analysed the model and wrote the paper.

Competing interests

We declare having no competing interests.

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Abstract

Sex is ancestral in eukaryotes. Meiotic sex differs from bacterial ways of exchanging genetic material by involving the fusion of two cells. We examine the hypothesis that fusion evolved in early eukaryotes because it was directly beneficial, rather than a passive side-effect of meiotic sex. We assume that the uptake of (proto)mitochondria into eukaryotes preceded the evolution of cell fusion, and that Muller's ratchet operating within symbiont lineages led to the accumulation of lineage-specific sets of mutations in asexual host cells. We examine if cell fusion, and the consequent biparental inheritance of symbionts, helps to mitigate the effects of this mutational meltdown of mitochondria. In our model, host cell fitness improves when two independently-evolved mitochondrial strains co-inhabit a single cytoplasm, mirroring mitochondrial complementation found in modern eukaryotes. If fusion incurs no cost, we find that an allele coding for fusion can invade a population of non-fusers. If fusion is costly, there are two thresholds. The first describes a maximal fusing rate (probability of fusion per round of cell division) that is able to fix. An allele that codes for a rate above this threshold can reach a polymorphic equilibrium with non-fusers, as long as the rate is below the second threshold, above which the fusion allele is counterselected. Whenever it evolves, fusion increases the population-wide level of heteroplasmy, which allows mitochondrial complementation and increases population fitness. We conclude that beneficial interactions between mitochondria are a potential factor that selected for cell fusion in early eukaryotes.

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Introduction

The origin of sex in eukaryotes is a billion-year-old mystery. Phylogenetic studies and comparative genomics allow us to deduce that LECA, the Last Eukaryotic Common Ancestor that lived 1.0 to 1.6 billion years ago (Eme *et al.*, 2014), already engaged in sex (Schurko & Logson, 2008; Goodenough & Heitman, 2014; Speijer *et al.*, 2015). Sex for LECA, as for most of its descendants, can be defined as the fusion of two haploid cells, and the coming together of their nuclear chromosomes, to form a zygote (Lehtonen & Kokko, 2014). Meiosis then allows alternation between diploid and haploid phases and adds chromosomal recombination to the mix. Eukaryotic cells typically do not engage in sex every generation. Instead, multiple mitotic divisions take place between sexual bouts in both unicellular and multicellular organisms (Green & Noakes, 1995; Dacks & Roger, 1999).

It is difficult to reconstruct how LECA evolved its sexual cycle, as opposed to prokaryotic means of genetic exchange such as conjugation, transformation or transduction (Lehtonen & Kokko, 2014). The common ancestor of LECA and its closest extant prokaryotic lineage lived long before LECA itself arose (up to one billion years, Dacks *et al.*, 2016), obscuring the order in which all defining features of eukaryotes were gained (including linear chromosomes, a nucleus, a cytoskeleton, mitochondria, and meiotic sex, Koonin & Yutin, 2010, Cavalier-Smith, 2010). Despite several false alarms, no intermediate forms are known to have survived to document the timeline of eukaryogenesis (Dacks *et al.*, 2016; Zachar & Szathmáry, 2017). Additionally, the origin of sex was probably a response to different selective pressures than the ones responsible today for its maintenance (Hartfield & Keightley, 2012; Lehtonen *et al.*, 2016), and those past selective pressures can only be inferred, not observed. Here we consider whether cytoplasmic fusion and mitochondria might be key features of eukaryotic evolution that prepared the ground for the evolution of

sex. As such, we assume that mitochondrial symbionts were acquired before the evolution of sex (which is still debated: Koonin & Yutin, 2010; Pittis & Gabaldón, 2016a; Martin *et al.*, 2017; Degli Esposti, 2016; Pittis & Gabaldón, 2016b).

Previous authors have proposed that the acquisition of mitochondria selected for the evolution of sex. Two verbal models focus on the genetic benefits of sex. Lane (2011) considers sex as a way for the host cell to maintain genome integrity against disruptions caused by mitochondria — either due to the reactive oxygen species generated by mitochondrial metabolism, or due to bombardment of the host's genome by mitochondrial DNA (Martin & Koonin, 2006). Havird *et al*. (2015) argue that sex aided mitonuclear coevolution, which was necessary to keep the symbiosis functional. Mathematical modelling has, so far, focused on the evolution of the seemingly simpler step of cell fusion (Radzvilavičius & Blackstone, 2015; Radzvilavičius, 2016a). Fusion is an intriguing phenomenon in its own right. It requires that the cells dissolve their cell walls and membranes, and it potentially enables transmission of cytoplasmic infections. It could therefore be seen as an inefficient way to exchange nuclei – especially since genetic material *can* be exchanged without requiring cellular fusion or much cytoplasmic mixing of the two partners, as exemplified in prokaryotes by use of "sex pili" during conjugation (Schröder & Lanka, 2005; Cabezón *et al.*, 2014), and in eukaryotes by the formation of cytoplasmic bridges in ciliates (Adoutte & Beisson, 1972).

Nevertheless, fusion is a prerequisite for nuclear recombination in the vast majority of eukaryotes and is likely ancestral. This raises the question of whether fusion itself might have been selected for in early eukaryotes before becoming coopted as an integral part of the sexual cycle. A key issue is that mitochondrial inheritance cannot be assumed to have been uniparental from the start (Birky, 1995; Radzvilavičius & Blackstone, 2015; Radzvilavičius, 2016a), despite uniparentality being virtually universal in modern sexual eukaryotes, where

elaborate machinery is required to enforce it (see Breton & Steward, 2015, for a discussion of the very few exceptions). In asexual cell lineages, uniparentality also occurs by default. However, any transitional state towards sex that involves cell fusion should *a priori* lead to cytoplasmic mixing and biparental inheritance of mitochondria (Birky, 1995).

Since eukaryotic sex involves cell fusion, it appears necessary to consider two transitions in mitochondrial inheritance: from uniparental to biparental (when fusion first evolved), and back to uniparental. While the latter transition has been the object of significant theoretical effort in order to explain the prevalence of uniparentality (Hastings, 1992; Law & Hutson, 1992; Godelle & Reboud, 1995; Hadjivasiliou *et al.*, 2013; Christie *et al.*, 2015; Christie & Beekman, 2017a, b; Radzvilavičius *et al.*, 2017a), the former has largely escaped attention. The transient evolution of biparentality can be thought of in three different ways: at this point of eukaryotic evolution when the transition occurred, biparental inheritance could have been (i) neutral, (ii) deleterious, but it evolved because other benefits of fusion overrode its costs, or (iii) beneficial and, being selected for in its own right, had the potential to drive the evolution of cell fusion. The last possibility (iii), which is the one this paper investigates, implies that selection pressures changed throughout eukaryote evolution, eventually making biparental inheritance counter-selected, as shall be addressed in the discussion.

Recently, the evolution of cell-cell fusion and cytoplasmic mixing has been modelled by Radzvilavičius & Blackstone (2015) and Radzvilavičius (2016b), who studied the spread of an allele triggering fusion in a population of otherwise clonal eukaryotes. In these models, mitochondria can be of one of two types: wild-type or mutated (with ongoing mutation from the former to the latter state). The fitness of a host cell depends on the number of mutated symbionts it possesses. Frequent fusion homogenizes the content of cells in the population, so that they all contain an intermediate proportion of mutated mitochondria (leading to high

intra- and low inter-cellular variance). This can be beneficial for a cellular lineage, but only when maintaining a mediocre cytoplasm over generations is better than producing some offspring with high and some with low mitochondrial mutation load. A necessary, but not sufficient, condition for fusion to evolve was that the deleterious impact of an additional defective mitochondrion increases with the number of mutated symbionts already in the cell. Overall, the parameter space in which cell fusion evolves in these models is narrow, and the selective advantage is small, suggesting that controlling the number of mutated mitochondria within the cytoplasm might not have been the one major driving force behind the evolution of cell fusion.

In our model, we assume two different mitochondrial lineages. In contrast with models discussed above, we assume both lineages to be in a mutated state, but each with a different set of deleterious mutations. A cell homoplasmic for one type of mitochondria suffers the full phenotypic consequences of its mutated mitochondria and the associated fitness costs (Fig. S1A,D,E). A heteroplasmic cell, containing a mixture of both mitochondrial types, enjoys the benefits of complementation, and if there are sufficient numbers of both types present, the deleterious mutations are not expressed at the cell level (Fig. S1B,C,F). Complementation between mitochondrial strains carrying different mutations has been reported in extant eukaryotes (Takai *et al.*, 1999; Gilkerson *et al.*, 2008; Ma *et al.*, 2014), even though one study also reported negative interactions happening between two otherwise healthy mitochondrial strains (Sharpley *et al.*, 2012). Why complementation resulting from biparental inheritance could have played an important role at the onset of the symbiosis while being virtually irrelevant nowadays is addressed in our discussion. Note that we use the word complementation in a broad sense (following Sato *et al.*, 2009) to include effects of masking (non-expression of a mutated allele thanks to the presence of its wild-type counterpart, reviewed in Rossignol *et al.*, 2003) as well as complementation *sensu stricto*

(restoration of a mitochondrial function when two strains carrying mutations on different genes related to this function are put together, e.g. Takai *et al.*, 1999; Gilkerson *et al.*, 2008; Nakada *et al.*, 2002; Nakada *et al.*, 2009; Ma *et al.*, 2014). We show that cell-cell fusion can evolve under complementation, since fusion enables a cell to maintain heteroplasmy, offering a way to restore a fully functional cytoplasm.

Methods

General design

We assume an infinite population of haploid (proto)eukaryotic cells whose fitnesses depend on their (proto)mitochondria. The host cells have a life cycle that consists of viability selection, cell fusion (whether a cell fuses depends on genotype and population composition, see below), and asexual reproduction by mitotic division.

We consider a starting point where cells are asexual and fusion is absent. Cells are homoplasmic for one of two possible mitotypes, A or B. As both mitotypes A and B have their own set of deleterious mutations, a heteroplasmic cell, i.e. with a cytoplasm consisting of a mixture of A and B, is assumed to have higher survival than either type of homoplasmic cell, i.e. with A or B alone, due to mitochondrial complementation (Rossignol *et al.*, 2003, see Fig. S1 for details). We then introduce a mutant allele that causes its carrier to fuse with another randomly selected cell in a proportion r of its reproductive cycles, and examine whether the benefits of heteroplasmy can overcome the costs of cell fusion. A mutant cell can initiate fusion with a non-mutant, consistent with unilateral fusion requirements possibly found in the gametes of early eukaryotes (Hernández & Podbilewicz, 2017). We run independent simulations that differ in the rate of fusion r expressed by the fusing genotype,

which is consistent with facultative sex in modern unicellular eukaryotes. We vary the reproductive cost incurred by both partners of a fusion (which could for instance be a time cost), the shape of the complementation function, the number of mitochondria per eukaryotic cell, and the extent of mitochondrial turnover during a cell's lifetime (modelled by altering the variance in the cytoplasmic content of daughter compared to mother cells).

The simulation thereafter tracks the composition of the population in a deterministic manner, meaning that we omit drift. We monitor the evolution of frequencies of the cytoplasmic classes (i.e. classes of cells with a specific number of mitochondria of type A and B), as well as the frequency of the mutant allele coding for fusion in each class. We use f_t to denote the overall frequency of the mutant allele at generation t.

Each generation proceeds as follows.

Life cycle: 1. Viability selection

A cell's probability of surviving, φ, depends on the relative proportion of each of the two mitotypes present among its M mitochondria, following the description of the phenotypic threshold effect of mitochondrial mutations found in Rossignol *et al.* (2003; see also Fig. S1). For a cell with i mitochondria of type A among its M, we assume a complementation function

$$\varphi\left(\frac{i}{M}\right) = 1 - K\left(1 - 2\frac{i}{M}\right)^2 \quad (1).$$

where K is the survival probability difference between maximally heteroplasmic and homoplasmic cells (Fig. 1). Viability is highest when $i = \frac{1}{2}M$ (maximally heteroplasmic state), and lowest at the two possible homoplasmic states i=0 or i=M. Note that symmetry

implies that both mitotypes have a set of mutations impacting fitness with the same effect size. Some different complementation functions leading to qualitatively similar results are presented in the supplementary material (Fig. S4), including functions relaxing the assumptions that that the two mitotype have accumulated deleterious mutations with similar cumulated effect size (Fig. S10.

Life cycle: 2. Fusion

Prior to reproduction, a proportion r of mutant cells initiate fusion with a randomly selected partner in the population, mutant or not, with which they mix their cytoplasmic contents before separating again. Note that the proportion of cells that undergo fusion in the population differs from the frequency f of the mutant allele. This is because (i) a mutant cell only attempts fusion with probability $r \leq 1$, and (ii) a cell that does not attempt to fuse (mutant or not) might still be chosen as a partner by a cell that does (Table 1).

When two cells fuse, mix their cytoplasm, and separate again, the probability that one of the resulting cells inherits i mitochondria of type A follows the hypergeometric distribution (sampling without replacement):

$$D(i|M,k) = \frac{\binom{k}{i}\binom{2M-k}{M-i}}{\binom{2M}{M}} \quad (2).$$

where k is the total number of mitochondria of type A present post-fusion in the double cell, and M denotes the number of mitochondria per single cell.

Life cycle: 3. Reproduction

This stage is distinct from the fusion and fission above; all cells reproduce asexually regardless of whether they have participated in fusion before. However, cells that did engage in fusion (whether they initiated it or were simply chosen as partners) have decreased reproductive output compared to those that did not fuse: their relative

contribution to the next generation drops from 1 to 1–*c*. In an infinite population with deterministic dynamics, each cell generates a distribution of daughters with all possible cytoplasmic contents, which is then scaled to sum up to a contribution to the next generation of 1 or 1–*c*. The probability for a daughter cell to inherit a certain cytoplasmic content is determined by binomial sampling (i.e. with replacement) from the mother cell's content. Sampling with replacement is chosen to simulate mitochondrial turn-over and drift within the cytoplasm of the cell during its life. The following matrix gives the probability of obtaining a cytotype with *i* type A mitochondria among its *M*, from a parent cell with *k* mitochondria of that type among its *M*.

$$D_{ik} = d(i|M,k) = \binom{M}{i}\left(\frac{k}{M}\right)^{\mathrm{i}}\left(1-\frac{k}{M}\right)^{M-i} \quad (3).$$

Results obtained with sampling without replacement, i.e. a procedure leading to less variance in progeny content, can be found in the supplementary material (Figs S4–S9).

Simulations

A simulation starts with a population composed of 50% of cells homoplasmic for type A, and 50% of cells homoplasmic for type B mitochondria. These starting conditions yielded the same outcome as additional simulations where the starting point was a population of heteroplasmic cells at segregation-selection equilibrium (see more details below). A fusing allele is introduced at a low frequency (1%) among cells hosting one of the mitochondrial lineages. A simulation runs until the mutant allele has been lost (frequency $f < 10^{-8}$), has reached fixation ($f > 0.99$), or has reached a stable frequency ($\Delta f < 10^{-7}$ for 1000 generations). We run simulations for a range of fusion rates *r* to determine two thresholds, for each fusion cost *c* and number of mitochondria per cell *M*: the highest rate able to invade, as well as the highest rate able to reach a polymorphic equilibrium (beyond that rate, the fusing allele is

counter-selected and goes extinct). As each simulation leads to a deterministic outcome, the threshold locations can be narrowed down efficiently with the bisection method.

To study the impact of fusion on population parameters such as fitness mean and variance, and heteroplasmy mean and variance, we compare the results of a run where all cells use the highest rate of fusion that we found to be able to fix, to the results of simulations with the same parameters but with no cell fusion. In those reference populations, heteroplasmy exists but is maintained at an equilibrium distribution solely by the balance between selection and segregation. While we create these reference populations mainly as a conceptual tool to be able to isolate the role of fusion in maintaining heteroplasmy and population fitness, we note that they are not mere hypothetical constructs but can also arise naturally. In some of our evolutionary simulations where the fusion allele eventually goes extinct, fusion persists nonetheless long enough to mix cytoplasms and generate heteroplasmy, which is thereafter maintained by selection-segregation (Fig. S2, S3). Here, to generate such populations for comparison's sake, we artificially start them with only asexual cells with maximum heteroplasmy, and let them reproduce until the population has reached segregation-selection equilibrium where the distribution of cytoplasmic types in the asexual population is stable (this is possible because the model is deterministic). All simulations were implemented in R-3.4.3 (R Core Team, 2017).

Results

We find that a mutant allele causing cell fusion and mitochondrial reshuffling can invade a population of non-fusing cells. For each combination of number of mitochondria and cost of fusion *c*, there exists a maximum fusing rate that can be fixed in a population (Fig. 2, 3; S3, S4), and a maximum fusing rate that reaches a stable intermediate frequency in the

population (Fig. 3). Beyond the polymorphism threshold, the costs of fusion are incurred too frequently to outweigh its complementation-driven benefits, and the fusion-inducing allele goes extinct.

Unsurprisingly, higher fusion costs decrease the frequency of fusion that can fix. Without a cost, cells evolve to fuse every generation ($1–c = 1$, Fig. 2, S3, S4). The number of mitochondria M also impacts the profitability of fusion. For M = 4 and above, the higher the number of mitochondria, the lower the advantages of fusion. Since mitochondria are randomly segregated between daughter cells during cell division, heteroplasmic cells are more likely to generate homoplasmic daughters when they have few mitochondria than when they have many. This has consequences for the frequency of low-fitness, low heteroplasmy cells in the population (Fig. 4A), which are the cells benefitting the most from fusion (Fig. 4B). More cells with low heteroplasmy means more cells benefitting greatly from fusion, which translates to a higher expected benefit of fusion that is able to outweigh more severe costs.

This logic does not hold for very low numbers of mitochondria per cell, where random segregation is much more likely to produce homoplasmic cells. Here the benefits of fusion are easily outweighed by its costs. The rate of fusion required to maintain heteroplasmy is now so high that the associated costs become too severe. In other words, the problem of homoplasmy becomes too difficult to avoid, as random segregation operates too powerfully.

Whenever it evolves, fusion increases the average fitness of a population and the average population heteroplasmy (Fig. S5, S6). For low numbers of mitochondria, this fitness gain is associated with an increase in fitness variance and heteroplasmy variance in the population (Fig. 5, Fig. S7, S8). This is because an asexual population with few mitochondria is composed mainly of homoplasmic cells with low fitness (Fig. 4), and the evolution of cell

fusion allows more of the fitter, heteroplasmic types to be maintained (Fig. 5B, S6). For high numbers of mitochondria, the fitness gain is associated with a decrease in fitness variance. Here, an asexual population can already maintain high levels of heteroplasmy and fitness (Fig. 4B), and fusion allows further narrowing of its distribution around that optimum.

Our results appear qualitatively robust regardless of the precise shape of the complementation function, but the exact parameter space in which fusion can evolve depends on our choice of this function and its parameters (see Figs. S4 and S10 for instances where fusion is more, or less, likely to evolve than in the main example). Prospects for the invasion of fusion become weaker when sampling occurs without replacement (Fig. S5–S9), as this creates less variance between the cytoplasmic content of mother and daughter cells and improves an asexual lineage's ability to remain heteroplasmic. An asymmetric fitness function also reduces the parameter space in which fusion evolves (Fig. S10), because in many cases the mitotype with less severe mitochondrial mutations will fix in the population before fusion can spread. Finally, we find that the benefits of cell fusion, measured as the cost that fusion can carry and still evolve (as seen on Fig. 2), are predicted well by the initial fitness advantage a fusing mutant gets in a population of non-fusers at segregation-selection equilibrium (Fig. S9).

Discussion

Mitochondrial complementation can select for cell fusion

We explored the possibility that cell fusion — nowadays closely intertwined with meiotic sex — could have initially evolved to enable complementation between different mitochondrial strains in the same cytoplasm. Our model explores the conditions under

which a mutation triggering occasional fusion spreads in a population of protoeukaryotes. Cell fusion is beneficial because it counteracts the effects of random segregation and therefore enhances heteroplasmy in daughter cells (Radzvilavičius, 2016b). In line with the general statement that rare sex may often yield a better cost-benefit balance than obligate sex (Burke & Bonduriansky, 2017), we find that fusing every generation is only selected for if fusion is cost-free; costly fusion leads to it being employed cyclically, with several rounds of clonal reproduction taking place between bouts of fusion.

Occasional sex together with long periods of asexual reproduction is common among extant unicellular eukaryotes (Dacks & Roger, 1999; Nieuwenhuis & James, 2016), and is also expected from theoretical models on the maintenance of sex and recombination (Green & Noakes, 1995; Burke & Bonduriansky, 2017), though for reasons different from the ones modelled in this paper, since mitochondrial inheritance is nowadays uniparental. For instance, the order of magnitude of the frequency of sex has been estimated as once every 10^2 to 10^5 generations in the marine unicellular *Pseudoperkinsus tapeti* (Marshall & Berbee, 2010), every 10^3 in the wild yeast *Saccharomyces paradoxus* (Tsai *et al*., 2008), and every 10 to 10^4 generations in the budding yeast *S. cerevisiae (*Ruderfer *et al*., 2006; here the estimate is of the outcrossing rate). In our model, the descendants of a heteroplasmic cell become progressively more homoplasmic over multiple clonal generations, reaching a switching point after which the benefits of fusion exceed the (fixed) costs. While we do not assume cells to be able to monitor their own heteroplasmy, frequencies of fusion that can evolve reflect the speed at which a mitotically-dividing cellular lineage loses heteroplasmy. This speed is increased by the variance between the cytoplasmic content of a mother and its daughter. This explains our finding that the lower the number of mitochondria per cell, the higher the fusion frequency that can be selected for: a clonal lineage becomes homoplasmic faster when there are only few mitochondria. Additionally, a procedure reducing the

variance between mother and daughters during division decreases the optimal fusion frequency (Fig. S4).

Our model uses a range for the numbers of mitochondria consistent with modern unicellular eukaryotes: Okie *et al.* (2016) gathered data for 23 species, where they found that the number of mitochondria scales with cell size, and that 90% of the species had less than 260 mitochondria per cell, with a median number of 43 (range of 2-17'700, Jordan Okie, personal communication).

How do our results relate to others'?

One of our results is that the benefits of fusion tend to decrease with the number of mitochondria per cell. This is congruent with earlier results obtained by Radzvilavičius & Blackstone (2015) and Radzvilavičius (2016b), albeit from a different standpoint. There, mitochondria are modelled as being either cooperative, or selfish with a replication advantage, and the authors investigate whether cell fusion can spread. Fusion is a double-edged sword in this case: it can allow a cell to mitigate its number of selfish mitochondria, but also favours the transmission of faster replicating selfish mitochondria. Fusion in this setting can evolve if the replication advantage enjoyed by selfish mitochondria is low, and fusion frequency is high. Like in our model, lower numbers of mitochondria per cell (50 vs 200 or 20 vs 50 in their case) increases the likelihood that cell-cell fusion evolves. The reason is that for small numbers of mitochondria, segregation generates higher variance between daughter cells, and allows a stronger purifying selection to operate, constraining the spread of selfish symbionts, and making fusion a safer process.

Our main assumptions contrast in two ways with other models. First, discussions of the evolution of cell fusion (e.g. Lane, 2012) typically do not include the possibility of complementation between mitochondrial lineages. Second, we assume that mitochondrial

lineages trapped in different clonal lineages of asexual protoeukaryotes diverge, which contrasts with a coevolutionary scenario (between the nucleus and mitochondria) presented by Havird *et al.* (2015). Their verbal model is placed in a setting where tight interactions between nuclear and mitochondrial proteins have already evolved, and assumes that a high rate of mitochondrial mutation selects for nuclear genomes to increase their rate of adaptation, which they achieve by recombining (an argument akin to the Red Queen hypothesis). Without a mathematical model, it is difficult to evaluate if nuclear adaptation to a given mitochondrial background is facilitated or impaired by shuffling alleles between cells, if each lineage has accumulated different mutations and potentially adapted to them.

List of assumptions and limitations

Our simple proof of principle that mitochondrial complementation could have played a role in the evolution of cell fusion relies on a number of assumptions, which, if proven unlikely in the future, can be used to reject complementation as a potential contributor to the origin of sex. It is also worth noting that our model focuses on the origins of cell fusion, not on its maintenance; hence its assumptions are tailored to fit the onset of eukaryogenesis rather than any selection pressures acting in its later stages. Indeed, biparental inheritance and maintenance of heteroplasmy, the cornerstones of our model, are clearly not selected for in extant eukaryotes.

The three main assumptions we detail below relate to the timing of endosymbiosis, the mechanistic potential for complementation, and the genetic potential for complementation, assumed to have changed through eukaryogenesis.

First, our model assumes that the acquisition of the bacterium relative to alpha-proteobacteria that later became the mitochondrion happened early in eukaryogenesis, preceding the evolution of cell fusion and sex. An early onset of the symbiosis clearly has the

potential to dramatically affect the subsequent evolution of the host, and it has been argued by some to be the driving force behind eukaryogenesis (Lane, 2011; Martin *et al.*, 2016; but see Cavalier-Smith, 2010). Still, the "mito-early vs mito-late" debate has yet to be resolved unambiguously (Keeling, 2014; Pittis & Gabaldón, 2016a; Martin *et al.*, 2017; Degli Esposti, 2016; Pittis & Gabaldón, 2016b). More specifically, our model requires that fusion evolved at a time when coadaptation was sufficiently advanced for mutations in the symbiont to reduce the fitness of the host.

A second major assumption of the model is the coexistence of mitochondrial lineages with different deleterious mutations. Muller's ratchet, the irreversible accumulation of deleterious mutations in asexual genomes, is typically studied by tracking the dynamics of the loss of the least-mutated class – that is to say, by focusing on the number of mutations, not their identity (e.g. Bergstrom & Pritchard, 1998, Metzger & Eule, 2013, Christie & Beekman, 2017a, Radzvilavičius *et al.*, 2017a, in mitochondria; though see Gordo *et al.*, 2002, for a model of neutral genetic diversity in a ratchet setting). However, a mutational class (i.e. all individuals harbouring a given number of mutations) can comprise different lineages carrying different mutations. If the ratchet has led to the establishment of separate mitochondrial lineages, with different sets of mutations, but leading to comparable declines in host fitness, our process of host fitness restoration through complementation becomes conceivable; if the ratchet operates differently, our mechanism may work less well (Fig. S10). Note that while we have used accumulation of deleterious mutations as our conceptual framework, the complementation function we used can also be reformulated in terms of beneficial mutations: in the absence of recombination between organelles, the only way for a host to enjoy the combined effects of two beneficial mutations that arose on different mitochondrial lineages is to harbour both lineages simultaneously (Park & Krug, 2007, but

see Christie & Beekman, 2017a, on the benefits of uniparental inheritance to circumvent clonal interference, by increasing the fixation rate of beneficial mitochondrial mutations as they arise).

Third, our hypothesis relies on complementation (*sensu lato*, i.e. including masking) being possible and of sufficient efficiency between early mitochondria. It is still poorly understood how mitochondrial complementation occurs in modern organisms, but the fusion/fission cycles of mitochondria appear to play a role (Gilkerson *et al.*, 2008). Such mitochondrial dynamics seem to be a common feature of extant eukaryotes: it is found in organisms as varied as yeast (Rafelski, 2013), animals (Chan, 2006), amoebozoa (Schimmel *et al.*, 2012) and plants (Arimura *et al.*, 2004; Seguí-Simarro *et al.*, 2008). Still, fusion and fission are not behaviours displayed by eubacteria (Wagner *et al.*, 2017), which suggests a derived origin. However, metabolic complementation has also been found between different bacterial endosymbionts sharing an insect host (Rao *et al.*, 2015), indicating that complementation is possible in nascent endosymbioses.

Proposing mitochondrial complementation as a positive outcome of fusion is perhaps contentious, since the near-ubiquity of uniparental inheritance suggests that cytoplasmic mixing is somehow detrimental. Direct deleterious interactions between mitolines would indeed lead to the evolution of uniparental inheritance (Christie *et al.*, 2015), but are not required for that, as shown by a variety of models based on other processes (Hastings, 1992; Law & Hutson, 1992; Godelle & Reboud, 1995; Hadjivasiliou *et al.*, 2013; Christie & Beekman, 2017b; Radzvilavičius *et al.*, 2017a). Experimentally, direct detrimental interactions have only been clearly reported in one study to our knowledge (Sharpley *et al.*, 2012 in mice), although some confusion might result from the use of the phrase "deleterious heteroplasmy" in biomedecine. It refers to situations where a deleterious mutant only starts to negatively impact the phenotype of a cell after its frequency exceeds the threshold beyond which it is

no longer masked by healthy mitochondria (Rossignol et al., 2003). Importantly, the phrase does not refer to any negative interaction between mitochondrial strains.

Positive interactions, on the other hand, have been reported somewhat more widely, both between closely related strains within a patient's cells, and between diverged strains artificially put together in the lab. Complementation *sensu stricto* has been found in *Drosophila,* humans and mice (Takai *et al.*, 1992; Nakada *et al.*, 2002; Gilkerson *et al.*, 2008; Nakada *et al.*, 2009; Sato *et al.*, 2009; Ma *et al.*, 2014; but see Enrìquez *et al.*, 2000 for an argument on the rarity of the phenomenon), and masking is a well-known phenomenon in the medical literature, where a *de novo* deleterious mutation often starts impacting the phenotype only after exceeding a certain prevalence threshold within the cells (Rossignol *et al.*, 2003). Finally, the fitness benefits of heteroplasmy do not need to be very high for fusion to be selected for (Fig. S4B, K=0.1).

Biparental inheritance: from beneficial to detrimental?

When fusion evolved in eukaryotic cells, mitochondrial inheritance switched from uniparental to biparental, only to subsequently revert back to uniparental. Theory has so far mainly focused on explaining the second transition. In this paper, we focused on the first one. We explored the possibility that biparental inheritance was originally selected for, and drove the evolution of fusion, as opposed to fusion being directly selected for and biparental inheritance arising only as a by-product. The mechanism we tested was complementation between mitochondrial strains, and we showed that it could indeed have led to the evolution of fusion.

Why, though, would complementation have been particularly relevant for early eukaryotes and not modern ones? What changed to eventually make biparental inheritance selected against? We propose three arguments, related to the decline of the mutation rate, to the

reduction in the number of mitochondrial genes and their relocation into the nucleus, and to the invention of recombination that made the maintenance of heteroplasmy obsolete.

The genome of the protomitochondrial symbiont soon after the beginning of the symbiosis was large compared to that of modern mitochondria (Gray *et al*., 2001). It was also subject to a high mutation pressure due to poorly controlled oxidative phosphorylation and its mutagenous by-products (Hörandl & Hadacek, 2013; Speijer, 2014), and was probably evolving within a small population of protoeukaryotes. Such factors can lead to the rapid accumulation of different mutations trapped in different cellular lineages, setting the stage for our model: the only way to recover functional copies of the mutated genes was by bringing them together in one cytoplasm, resulting in a large fitness advantage to fusing cells.

Maintaining heteroplasmy by fusion is a short-term solution that cannot last indefinitely. At this stage, a functional mitochondrial genome could have been reconstituted if mitochondrial recombination was taking place at that time – a possibility that is difficult to verify at present. Homologous recombination between different molecules of mitochondrial DNA within a cell can occur in extant eukaryotes, as has been shown in some plants, fungi and animals (reviewed in White *et al*., 2008), although the taxonomic span and evolutionary history of that ability are not well assessed yet. Recombination has also been found among other bacterial symbionts of eukaryotes, e.g. *Wolbachia* (Baldo *et al*., 2005). Theoretical modelling shows that combining mitochondrial recombination with paternal leakage (i.e. moderate biparental inheritance) more efficiently counters Muller's ratchet in mitochondria than paternal leakage alone (Radzvilavičius *et al*., 2017b). Nevertheless, strict uniparental inheritance is yet more efficient at clearing deleterious mutations than paternal leakage with recombination, resulting in lower mutation loads within cells (Radzvilavičius *et al*., 2017).

Regardless of whether mitochondria do – or did – recombine, nuclear genes clearly do. Maintaining heteroplasmy for the purpose of complementation can become obsolete through the migration of most mitochondrial genes (or gene functions) into the nucleus, together with the evolution of nuclear recombination. In modern eukaryotes, fewer than 70 core proteins and RNAs are still encoded within the mitochondrion (Gray *et al*., 2004), while all other proteins (from ten to a hundred times as many) involved in mitochondrial function are encoded in the nucleus (Bousette *et al*., 2009; Boengler *et al*., 2011; Gray, 2015). The few genes left in mitochondria are highly conserved and appear to be under strong purifying selection (Mamirova *et al*., 2007; Popadin *et al*., 2012; Allen, 2015), probably due to the dangers associated with dysfunctional mitochondria.

The evolution of biparental and uniparental inheritance solve two different problems. The first transition, according to the hypothesis explored in our model, concerns organisms with a history of being clonal, currently in the process of domesticating a symbiont, and comprising of different cellular lineages losing different symbiotic functions due to high mutation rates and low population sizes. Under those conditions, biparental inheritance allows quick recovery of those functions via complementation. The second transition, on the other hand, presumably took place in sexual organisms in which most of the mitochondrial functions had been taken over by the nucleus, leaving mitochondrial genomes small, streamlined, and homogeneous. Such organisms experience a set of novel problems: how to best protect their established mitochondrial genomes against mutation (Radzvilavičius *et al*., 2017), to increase mito-nuclear co-adaptation (Hadjivasiliou *et al*., 2013), and to avoid the spread of selfish organelles (Hastings, 1992, Law & Hudson, 1992, Hadjivasiliou *et al*., 2013).

Conclusion

Our model is a proof-of-principle for a potential evolutionary pathway taking protoeukaryotes, hosting protomitochondria, from a clonal life cycle to a life cycle involving cell-cell fusion. This endpoint is still far removed from the putative state of our Last Common Eukaryotic Ancestor. Nuclear sex, uniparental inheritance, and a small and streamlined mitochondrial genome still had to evolve — which has been the focus of most models of eukaryogenesis. Cell fusion itself, however, remains a puzzle. Why mix cytoplasm, allowing biparental inheritance of mitochondria, if biparental inheritance is counterselected in extant organisms, and mechanisms allowing transfer of genetic material without cytoplasmic mixing were possible? Our model suggests mitochondrial complementation could have played a role.

Table 1

	Mutant allele	Resident allele	total
Undergoes fusion	$f_t r + f_t (1 - r)(f_t r)$	$(1 - f_t)(f_t r)$	$2 f_t r - f_t^2 r^2$
Does not undergo fusion	$f_t (1 - r)(1 - f_t r)$	$(1 - f_t)(1 - f_t r)$	$1 - (2 f_t r - f_t^2 r^2)$
total	f_t	$(1 - f_t)$	1

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Table & Figure legends

Table 1 Composition of the population at generation *t* (f_t is the frequency of the mutant allele, ($1–f_t$) the frequency of the resident allele, and r the rate of fusion of the mutants). When engaging in fusion, mutants randomly select another cell in the population, irrespective of its genotype.

Fig. 1 Complementation function. Unless stated otherwise, it is the function used throughout the paper. It follows Eqn 1 with $K = 0.3$.

Fig. 2 Fixation success of a fusing allele. The logarithmic colour scale denotes the highest rate of fusion able to fix in a population, for a given combination of mitochondria number and relative reproductive success of fusers. The lowest rate tested in simulations was 0.005.

Fig. 3 Too frequent fusion is counter-selected. The three panels have identical parameter values for c and represent three horizontal "slices" of Fig. 2 according to the number of mitochondria. For each combination of mitochondrial number and relative success of fusers, there exists a maximum fusing rate that can reach fixation (green area), and a maximum fusing rate that can reach a stable intermediate frequency in the population (pink area). Above that rate, fusion is selected against and disappears. The higher the number of mitochondria, the smaller the polymorphic and fixation areas.

Fig. 4 A population of asexual eukaryotes sets the stage for the evolution of fusion. a-c: The stable distribution of cytotypes that is reached at equilibrium in an asexual population (no fusion). The equilibrium is attained when random segregation (which tends to erode heteroplasmy) and natural selection (which eliminates homoplasmic cells) reach a balance. In the case of $M = 2$ (a), random segregation is too strong for any heteroplasmic lineage to be maintained, despite the higher fitness of heteroplasmic cells. d-f: The fitness benefit (expected viability) enjoyed by a mutant cell of a specific mitochondrial class, were it to fuse with a random partner. The red line indicates no relative advantage compared to a non-fuser. The more homoplasmic a cell, the more it would benefit from fusing, but to which extent depends on the population composition. By combining figures a-c and d-f, one can calculate the population average for the potential fitness advantage gained by fusing; it equals 1.054, 1.067, 1.029, for $M = 2$, 10 and 50, respectively, and matches well (i.e. is a good indication of) the fusing allele's invasion potential (Fig. S9).

Fig. 5 Fusion impacts variance both in fitness (a) and heteroplasmy (b) within the population. To produce (a), the heteroplasmy of a cell was calculated as $1 - |\ 1 - 2i/M\ |$, with i the number of mitochondria of type A among the M in the cytoplasm of that cell. Therefore it ranges from 0 (homoplasmic, i.e. the cell contains either 0 or 100% of type A mitochondria) to 1 (maximally heteroplasmic, i.e. the cell contains 50% of type A and 50% of type B mitochondria). "+"signs indicate when variance increased compared to what it was in the asexual population; variance decreased where there is no sign. Mean fitness and heteroplasmy increased everywhere due to fusion (Fig. S5).